## POLYPEPTIDE, POLYPEPTIDE MIXTURE AND EMULSIFIER

Publication number: JP11221024 (A)

Publication date: 1999-08-17

Inventor(s): TSUMURA KAZUNOBU; KUGIMIYA WATARU

Applicant(s): FUJI OIL CO LTD

Classification:

- international: A23L1/035; A23J3/16; A23J3/34; A61K8/06; A61K8/64; A61K47/42; B01F17/30;

C07K14/42; A23L1/03; A23J3/00; A61K8/04; A61K8/30; A61K47/42; B01F17/30; C07K14/415; (IPC1-7): A23J3/16; A23J3/34; A23L1/035; A61K7/00; A61K47/42;

B01F17/30; C07K14/42

- European:

Application number: JP19980025257 19980206 Priority number(s): JP19980025257 19980206

#### Abstract of JP 11221024 (A)

PROBLEM TO BE SOLVED: To obtain an emulsifier with excellent emulsifying power, enabling a stable emulsion to be prepared in the field such as of foods, cosmetics and medicines and the like. SOLUTION: This emulsifier consists of/or contains a polypeptide with a molecular weight of &It;=8,000 (determined by SDS-polyacrylamide electrophoresis) having an N-terminal amino acid sequence: Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp- Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe which is obtained, for example, by making a protease act on soybean &beta -conglycinin.

Data supplied from the esp@cenet database — Worldwide

## (19)日本国特許庁(JP)

# (12) 公開特許公報(A)

(11)特許出願公開番号

## 特開平11-221024

(43)公開日 平成11年(1999)8月17日

(51) Int.Cl. <sup>6</sup>		識別記号		FΙ							
A 2 3 J	3/16			Λ2	3 J	3/16					
	3/34					3/34					
A 2 3 L	1/035			A 2 :	3 L	1/035					
A 6 1 K	7/00			A 6	1 K	7/00				N	
	47/42					47/42	7/42 H				
			審查請求	未前求	請求	項の数 4	OL	(全	4	頁)	最終頁に続く
(21)出顧番号	+	特願平10-25257		(71)	出願人	00023	6768	-			
						不二	设油株式	会社			
(22)出顧日		平成10年(1998) 2月6日			大阪府大阪市中央区西心斎橋2丁目1番5						
						号					
				(72)	発明者	1 津村	和伸				
				茨城県筑波郡谷和原村絹の台4丁目3番地							
						不二	二製油株	式会社	生つ	くば	研究開発センタ
						一内					
				(72)	発明者	<b>釘宮</b>	渉				
						<b>茨城</b> !	茨城県筑波郡谷和原村絹の台4丁目3番地				
						不二	二製油株	式会社	土つ	くば	研究開発センタ
						一内					

## (54) 【発明の名称】 ポリペプチド、ポリペプチド混合物及び乳化剤

## (57)【要約】

【課題】食品をはじめ化粧品、医薬品などの分野に於いて、乳化力に優れ、安定なエマルションを調製できる乳 化剤を提供すること。

【解決手段】例えば、大豆β-コングリシニンにプロテアーゼを作用させて分子量8000以下(SDS-ポリアクリルアミド電気泳動法による)、N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-のポリペプチド、該ペプチドを含む乳化剤を得る。

## 【特許請求の範囲】

【請求項1】N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gl y-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8000以下のポリ ペプチド。

【請求項2】N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Glu-Leu-Ala-Phe-で分子量8000以下のポリペプチドを30%以上含むポリペプチド混合物。

【請求項3】N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gl y-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8000以下のポリペプチドを含む乳化剤。

【請求項4】N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gl y-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Glu-Leu-Ala-Phe-で分子量8000以下のポリペプチドを30%以上含む乳化剤。

## 【発明の詳細な説明】

#### [0001]

【発明の属する技術分野】本発明は、食品をはじめ化粧品、医薬品などの分野に於いて、エマルションの調製に際し利用することができるポリペプチド及び該ポリペプチドを用いた乳化剤に関する。

## [0002]

【従来の技術】大豆蛋白質は、高い栄養価ばかりでなく、乳化性、ゲル形成性、保水性等の様々な機能特性を備えていることから優れた食品素材として使用されてきた。従来、エマルションの製造には乳化剤としてグリセロール脂肪酸エステル、リン脂質、ソルビタン脂肪酸エステル、ショ糖脂肪酸エステル等の天然或いは合成乳化剤、或いは上記大豆蛋白をはじめ、乳蛋白、小麦蛋白等の蛋白質系乳化剤が使用されてきたが、これら一般に用いられている合成乳化剤は風味上の観点から食品エマルションの製造には適当ではなく、更に蛋白質系乳化剤は一般に乳化力が弱く、他の乳化剤を併用する必要があった。

【0003】近年、健康や安全を指向することから天然系乳化剤とりわけ蛋白質系乳化剤が注目され、乳蛋白では、特定のアミノ酸配列を有するポリペプチドを用いる方法(特開昭58-174232号公報)やバターミルクから得られる特定の画分(特開平8-51934号公報)などが知られており、小麦蛋白では、ある特定の部分分解物(特開昭64-14274号公報)などが知られている。大豆蛋白では、特定の条件で酵素分解する方法(特開昭56-26171号公報、特開昭57-16674号公報、特開平6-197788号公報)などが知られている。

## [0004]

【発明が解決しようとする課題】以上の実情に鑑み、本 発明は食品をはじめ化粧品、医薬品などの分野に於い て、乳化力に優れ、安定なエマルションを調製できる乳 化剤を提供することにある。

#### [0005]

【課題を解決するための手段】本発明者らは、上記課題を解決すべく鋭意研究した結果、大豆蛋白とりわけβ-コングリシニンにプロテアーゼを作用させて得られる、特定のポリペプチドが乳化力に優れ、安定なエマルションを調製できることを見い出し、本発明を完成するに至った。

【 O O O 6 】即ち、本発明は、N-末端アミノ酸配列がAs n-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8 O O O以下のポリペプチドである。又、本発明は、N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-As n-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8 O O O以下のポリペプチドを30%以上含むポリペプチド混合物である。又、本発明は、N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-As n-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8 O O O以下のポリペプチドを含む乳化剤である。N-末端アミノ酸配列がAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-で分子量8 O O O以下のポリペプチドを30%以上含む乳化剤が好ましい。

#### [0007]

【発明の実施の形態】本発明のポリペプチドは、大豆蛋白、特に所謂75画分である $\beta$ -コングリシニンにプロテアーゼを作用させて特定のポリペプチドとして得りことが出来る。又、アミノ酸合成により該ペプチドを合成することも出来る。 該ポリペプチドの分子量は8000以下 (SDS-ポリアクリルアミド電気泳動法による)が適当である。

【0008】又、そのN-末端アミノ酸配列が、Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pr o-Ser-Gln-Val-Glu-Leu-Ala-Phe-である。

【0009】又、本発明のポリペプチド混合物は、該ポリペプチドを30%以上含む。又、本発明の乳化剤は、上記ポリペプチドを含むものであり、その含有量は30%以上が適当であ。それ以下では乳化剤としての機能が劣る。

【0010】本発明のポリペプチド、ポリペプチド混合物及び乳化剤の製造法の一例を示す。

【0011】本発明のポリペプチドは、β-コングリシニンにプロテアーゼを作用させて得られる加水分解物に油脂を加えてエマルションを調製し、ポリペプチドをエマルションに濃縮させた後、該ポリペプチドが濃縮されたエマルションから油脂を除去して、該ポリペプチドを分離して製造することが出来る。

【0012】本発明のポリペプチドは、β-コングリシニンを含む大豆蛋白を基質として、プロテアーゼで分解することができる。ここで用いられるプロテアーゼは、

特に制限はなくパパイン、ペプシン、トリプシン、ズブ チリシン等が使用可能であるが、上記の性質を有するポ リペプチドを多く生成しうる酵素としてパパインが好適 に使用される。

【0013】本発明の乳化剤として用いるボリペプチドは、β-コングリシニンを含む大豆蛋白、好ましくは公知の方法 (例えばThanh and Shibasakiの方法; J. Agric. Food Chem., 24, 1117(1976)を例示できる)でβ-コングリシニンを分画或いは濃縮したものを基質とし、その0.5%~15%溶液に対して、プロテアーゼを基質固形分に対して、0.001~1%、好ましくは0.01~0.5%の範囲で添加され、20℃~90℃、好ましくは30~80℃に於いてpH=5~10、好ましくはpH=6~9で、5分~2時間、好ましくは10分~1時間反応させることで実施できる。尚、反応条件は、用いる酵素剤の至適温度、至適pHや安定pHおよび目的のポリペプチドの生成量から決定される。

【0014】酵素反応終了後、反応液から目的のポリペプチドを分離あるいは濃縮するには、pH分画、ゲルろ過、イオン交換クロマトグラフィー、等電点電気泳動、吸着法等の公知の分離手法を組み合わせて行うことが可能であり、必要あれば殺菌や乾燥を行う。

【0015】本発明の乳化剤は、上記ポリペプチドを含む(好ましくは30重量%以上)ものである。

【0016】本発明の乳化剤は、水と油脂を含み油滴が乳化分散された水中油型乳化組成物の調製に於いて有効に用いられる。水中油型乳化組成物の調製に用いられる油脂は、水中油型乳化を形成するものであれば特に限定されることなく種々のものを用いることができ、動植物由来の食用油脂例えば、牛脂、豚脂、魚油、大豆油、なたね油、ヤシ油、パーム油等及びこれらの硬化油、分別油、エステル交換油等、その他にシリコン油、香油、機械油、石油分留物等を挙げることができる。

【0017】本発明の乳化剤を用いて得られる水中油型乳化組成物の水相対油相の構成比は、特に限定されないが2/8~9/1で好ましくは3/7~8/2の比率(重量比)となるように調製することが好ましい。本発明のポリペプチド乳化剤の添加量は、乳化組成物の水相対油相の構成比や油相の種類により異なるが、その全量に対して通常0.01~10重量%好ましくは0.05~5重量%の範囲であることが好ましい。

【0018】本発明のポリペプチド乳化剤には、乳化を安定させる目的で従来より用いられている界面活性剤を併用することもできる。例えば、グリセロール脂肪酸エステル、リン脂質、ソルビタン脂肪酸エステル、ショ糖脂肪酸エステル、ポリオキシエチレンアルキルエーテル、ポリオキシエチレンソルビタンエステル、アルキルアミン誘導体、アルキルアルコール、アルキル糖誘導体等の天然或いは合成乳化剤を挙げることができる。また、乳化を補助して粘弾性を付与する目的で本発明のポリペプチド乳化剤以外の蛋白質や多糖類を添加してもよ

い。これら蛋白質の例としては、大豆蛋白、小麦蛋白、乳蛋白、卵白、卵黄、血液蛋白、魚肉蛋白、畜肉蛋白などの動植物由来の蛋白質やその分解物等を挙げることができる。また、公知のpH安定剤や蛋白溶解剤、例えば無機燐酸塩類、有機酸、EDTA等を使用することは任意である。

【0019】本発明のポリペプチド乳化剤を用いて乳化組成物の調製は、公知の乳化或いは均質化方法を用いて調製することができる。乳化の温度は、油相成分の性質に依存することがあるが、乳化剤の主成分であるボリペプチドの機能低下しない範囲で、一般に10~100℃、好ましくは20~90℃がよい。

【0020】本発明のポリペプチド乳化剤は、水中油型乳化組成物の調製に於いて、例えばクリーム類、マヨネーズ、ドレッシング、ペースト類などの乳化食品をはじめ、乳化香料、経腸栄養剤、化粧品乳液等に使用することができる。

## [0021]

【実施例】以下、実施例により本発明の実施様態を具体 的に説明するが、本発明がこれらによってその技術範囲 が限定されるものではない。

## 【0022】実施例1

Thanh and Shibasaki, J. Agric. Food Chem., 24, 1117(1976)の方法に従って得た大豆のβ-コングリシニンの2重量%溶液100mlをpH7、70℃に調整し、固形物重量当たり0.01%のパパイン (Sigma社製)を加え、30分酵素反応を行った。該反応液を100℃、5分加熱して酵素を失活させ、pH5に調整し、遠心分離した上清を凍結乾燥し、凍結乾燥物200mgを得た。凍結乾燥物の0.5重量%溶液(pH5)40mlに大豆油13.3mlを加え、超音波分散機で乳化物を調製した。該乳化物を遠心分離し、水層と乳化層に分離し、乳化層を回収した。該乳化層をクロロホルムーメタノール(2:1)溶媒で洗浄、風乾して、固形物(ポリペプチド混合物)70mgを得た。(収率3.5%)

【 O O 2 3 】上記ポリペプチド混合物をSDS-電気泳動で分析したところ、分子量5000乃至6000のポリペプチドが50%を占めるものであった(デンシトメーターによる定量)。SDS-電気泳動したゲルからこれら5000乃至6000のバンドをPVDF膜に転写し、プロテインシケンサーでこれらポリペプチドのN末端アミノ酸配列を調べたところ、どちらもAsn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-I1e-Ser-Gln-Ile-Pro-Ser-Gln-Val-Glu-Leu-Ala-Phe-から構成されるポリペプチドであった。

## 【0024】実施例2及び比較例1

実施例1で得たポリペプチド混合物の乳化力を濁度法にて評価した(J. Agric. Food Chem., 26,716(1978)の方法を参照)。即ち、0.05重量%溶液(pH7)1mlに大豆油0.25mlを加え、超音波分散機で乳化物を調製し、0.1%SDS溶液で500倍に希釈して溶液濁度を測定(500nmの吸光度)した。比較として、実施例1のパパイン反応液のpH

5上清乾燥物(以下比較例1)の乳化力を上記同様の方法で評価した。

【0025】乳化力(500nmの吸光度)

ポリペプチド混合物(実施例2)

0.35

pH5上清乾燥物

(比較例1)

0.18

ポリペプチド混合物は高い乳化力であった。

【0026】実施例3

実施例1で得たボリペプチド混合物のN末端アミノ酸配列に対応する23残基のボリペプチドをペプチド合成機にて合成した。この合成ペプチドを用い、実施例2の方法(但し、0.02重量%溶液(pH7)1mlを用いた以外は同様)で乳化力を評価したところ、乳化力(500nmの吸光度)は0.75であった。このように、上記N末端アミノ酸

配列に対応する23残基が高い乳化力に必須であった。

【発明の効果】本発明によれば、食品をはじめ化粧品、 医薬品などの分野に於いて、エマルションの調製に際し 利用することができるポリペプチド、ポリペプチド混合 物及び乳化剤が提供できる。

[0028]

【配列表】配列番号:1

配列の長さ:23 配列の型:アミノ酸 トポロジー:直鎖状 配列の種類:ペプチド

配列

Asn Phe Leu Ala Gly Ser Gln Asp Asn Val

1 5 10

Ile Ser Gln Ile Pro Ser Gln Val Gln Glu

11 15 20

Leu Ala Phe

21 23

## フロントページの続き

(51) Int. Cl. 6

識別記号

FI

B 0 1 F 17/30

CO7K 14/42

ZNA

BO1F 17/30 CO7K 14/42

ZNA

## **Machine translation JP11221024**

## **DETAILED DESCRIPTION**

- (19) **Publication country** Japan Patent Office (JP)
- (12)Kind of official gazettePublication of patent applications (A)
- (11)Publication No.JP, 11-221024, A
- (43) Date of Publication August 17, Heisei 11 (1999)
- (54) **Title of the Invention** Polypeptide, a polypeptide mixture, and an emulsifier
- (51)International Patent Classification (6th Edition)

À23J 3/16

3/34

A23L 1/035

A61K 7/00

47/42

B01F 17/30

C07K 14/42 ZNA

## FT

A23J 3/16

3/34

A23L 1/035

A61K 7/00 N

47/42 H

B01F 17/30

C07K 14/42 ZNA

Request for ExaminationUnrequested

The number of claims 4

Mode of ApplicationOL

**Number of Pages**4

(21) Application number Japanese Patent Application No. 10-25257

(22) Filing date February 6, Heisei 10 (1998)

(71)Applicant

**Identification Number**000236768

NameFUJI OIL Co., Ltd.

Address2-1-5, Nishi-Shinsaibashi, Chuo-ku, Osaka-shi, Osaka

(72)Inventor(s)

NameKazunobu Tsumura

**Address**4-3, Kinunodai, Yawara-mura, Tsukuba-gun, Ibaraki-ken Inside of FUJI OIL Tsukuba Research-and-development Center

(72)Inventor(s)

NameWataru Kugimiya

**Address**4-3, Kinunodai, Yawara-mura, Tsukuba-gun, Ibaraki-ken Inside of FUJI OIL Tsukuba Research-and-development Center

## (57) Abstract

**Technical problem** Excel in emulsification power in the field of cosmetics, drugs, etc. including foodstuffs, and provide the emulsifier which can prepare a stable emulsion.

**Means for Solution**Make protease act it on soybean beta-KONGU ricinine, and For example, 8000 or less (based on an SDS-polyacrylamide electrophoresis method) molecular weight, N-terminal-amino-acid arrangement Polypeptide of Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-, An emulsifier containing this peptide is obtained.

## Claim(s)

**Claim 1**N-terminal-amino-acid arrangement is Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Glu-Leu-Ala-Phe-, and it is with a molecular weight of 8000 or less polypeptide.

**Claim 2**N-terminal-amino-acid arrangement. By Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-, with a molecular weight of 8000 or less polypeptide. A polypeptide mixture included not less than 30%.

**Claim 3**N-terminal-amino-acid arrangement. An emulsifier which contains with a molecular weight of 8000 or less polypeptide by Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-.

**Claim 4**N-terminal-amino-acid arrangement. An emulsifier which contains with a molecular weight of 8000 or less polypeptide not less than 30% by Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-.

## Detailed Description of the Invention

0001

Field of the InventionIn the field of cosmetics, drugs, etc., this invention relates to

the emulsifier using the polypeptide which can be used when preparing an emulsion, and this polypeptide including foodstuffs.

#### 0002

**Description of the Prior Art**Soybean protein has been used as a food material outstanding from having various functional characteristics, such as not only a high nutritive value but emulsifiability, a gel plasticity, water retention, etc. Although nature, such as glycerol fatty acid ester, phospholipid, a sorbitan fatty acid ester, and sucrose fatty acid ester, a synthetic emulsifier, or the above-mentioned soybean protein was begun as an emulsifier for manufacture of the emulsion and protein system emulsifiers, such as lactalbumin and wheat protein, have been used conventionally, The synthetic emulsifier used for general **these** was not suitable for manufacture of the viewpoint on flavor to a foodstuffs emulsion, and also generally the protein system emulsifier had weak emulsification power, and needed to use other emulsifiers together.

**0003**Since it points to health or safety in recent years, it attracts attention from a natural system emulsifier division protein system emulsifier, and in lactalbumin. The specific fraction (JP,8-51934,A) etc. which are obtained from the method (JP,58-174232,A) of using the polypeptide which has a specific amino acid sequence, or butter milk are known, A certain specific partial decomposition product (JP,64-14274,A) is known for wheat protein. The method (JP,56-26171,A, JP,57-16674,A, JP,6-197788,A) of carrying out zymolysis on specific conditions, etc. are known for soybean protein.

## 0004

**Problem(s) to be Solved by the Invention**It is in this invention providing the emulsifier which is excellent in emulsification power in the field of cosmetics, drugs, etc. including foodstuffs, and can prepare a stable emulsion in view of the above actual condition.

## 0005

**Means for Solving the Problem**Specific polypeptide produced by making protease act on soybean protein division beta-KONGU ricinine is excellent in emulsification power, and finds out that a stable emulsion can be prepared, and this invention persons came to complete this invention, as a result of inquiring wholeheartedly that an aforementioned problem should be solved.

**0006**Namely, this invention, N-terminal-amino-acid arrangement is with a molecular weight of 8000 or less polypeptide in Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-. This invention, N-terminal-amino-acid arrangement. By Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Glu-Leu-Ala-Phe-, with a molecular weight of 8000 or less polypeptide. It is a polypeptide mixture included not less than 30%. This invention, N-terminal-amino-acid arrangement. It is an emulsifier which contains with a molecular weight of 8000 or less polypeptide by Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-. N-terminal-amino-acid arrangement. By Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-, with a molecular weight of 8000 or less polypeptide. An emulsifier included not less than 30% is preferred.

#### 0007

**Embodiment of the Invention**the polypeptide of this invention makes protease act on soybean protein and beta-KONGU ricinine which is what is called 7S fractions especially -- as specific polypeptide -- \*\*\*\* -- things are made. This peptide is also compoundable by amino acid synthesis. 8000 (based on an SDS-polyacrylamide electrophoresis method) or less are suitable for the molecular weight of this polypeptide.

0008The N-terminal-amino-acid arrangement is Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-

Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe-.

**0009**The polypeptide mixture of this invention contains this polypeptide not less than 30%. The emulsifier of this invention contains the above-mentioned polypeptide, not less than 30% of the content is suitable, and it is \*\*. The function as an emulsifier is inferior by less than it.

**0010**An example of the manufacturing method of the polypeptide of this invention, a polypeptide mixture, and an emulsifier is shown.

**0011**The polypeptide of this invention adds fats and oils to the hydrolyzate produced by making protease act on beta-KONGU ricinine, and prepares an emulsion, After making an emulsion condense polypeptide, fats and oils can be removed from the emulsion by which this polypeptide was condensed, and this polypeptide can be separated and manufactured.

**0012**The polypeptide of this invention can be decomposed by protease by using as a substrate soybean protein containing beta-KONGU ricinine. The protease used here is used as the enzyme which can generate many polypeptides which have the abovementioned character although there is no restriction in particular and it is usable in papain, pepsin, trypsin, subtilisin, etc., and papain is used suitably.

**0013**The polypeptide used as an emulsifier of this invention, Fractionation or the condensed thing is used as a substrate for beta-KONGU ricinine by soybean protein containing beta-KONGU ricinine and a desirable publicly known method (for example, method; J.Agric.Food Chem. of Thanh and Shibasaki, and 24 and 1117 (1976) can be illustrated), Substrate solid content is received in protease to a solution its 0.5% - 15%, 0.001 to 1%, it is preferably added in 0.01 to 0.5% of range, and in 30-80 \*\*, it is pH=6-9 preferably and 20 \*\* - 90 \*\* can be preferably carried out by pH=5-10 and making it react preferably for 10 minutes - 1 hour for 5 minutes - 2 hours. A reaction condition is determined from the optimum temperature of the enzyme agent to be used, optimal pH, stable pH, and the generated amount of the target polypeptide.

**0014**After the end of an enzyme reaction, in order to separate or condense the target polypeptide from reaction mixture, it is possible to carry out combining the publicly known separation techniques, such as pH fractionation, gel filtration, ion exchange chromatography, isoelectric focusing, and an adsorption process, and sterilization and desiccation with necessity are performed.

**0015**The emulsifier of this invention contains the above-mentioned polypeptide (preferably 30 % of the weight or more).

**0016**In preparation of the oil-in-water type emulsifying composition in which emulsification dispersion of the oil droplet was carried out including water and fats and oils, the emulsifier of this invention is used effectively. The fats and oils used for preparation of an oil-in-water type emulsifying composition, various things can be used without being limited especially if oil-in-water type emulsification is formed --edible oil and fat of animals-and-plants origin -- for example, Silicone oil, such as these hydrogenated oil, such as beef tallow, lard, fish oil, soybean oil, rape oil, palm oil, and palm oil, a judgment oil, and an ester interchange oil, perfumed oil, machine oil, a petroleum fraction, etc. can be mentioned.

**0017**Although the percentage in particular of the aqueous phase versus the oil phase of the oil-in-water type emulsifying composition obtained using the emulsifier of this invention is not limited, it is preferred to prepare so that it may become a ratio (weight ratio) of 3 / 7 - 8/2 preferably by 2 / 8 - 9/1. Although the addition of the polypeptide emulsifier of this invention changes with the percentage of the aqueous phase versus the oil phase of an emulsifying composition, or kinds of oil phase, it is preferred that it is usually 0.05 to 5% of the weight of a range preferably 0.01 to 10% of the weight to the whole quantity.

0018The surface-active agent conventionally used for the polypeptide emulsifier of

this invention in order to stabilize emulsification can also be used together. For example, glycerol fatty acid ester, phospholipid, a sorbitan fatty acid ester, Nature, such as sucrose fatty acid ester, polyoxyethylene alkyl ether, polyoxyethylene sorbitan ester, an alkylamine derivative, alkyl alcohol, and an alkyl sugar derivative, or a synthetic emulsifier can be mentioned. Protein and polysaccharide other than the polypeptide emulsifier of this invention may be added in order to assist emulsification and to give viscoelasticity. As an example of these protein, the protein of the animals-and-plants origin of soybean protein, wheat protein, lactalbumin, an egg white, an egg yolk, blood protein, fish meat protein, meat protein, etc., its decomposition product, etc. can be mentioned. It is arbitrary to use publicly known pH stabilizer and protein dissolution agent, for example, inorganic phosphate, organic acid, EDTA, etc.

**0019**Preparation of an emulsifying composition can be prepared using publicly known emulsification or the uniformity method using the polypeptide emulsifier of this invention. Although it may be dependent on the character of an oil phase ingredient, the temperature of emulsification is a range in which the polypeptide which is the main ingredients of an emulsifier does not carry out a depression, and, generally is preferably good. **of of 10-100** \*\* **20-90** \*\*

**0020**The polypeptide emulsifier of this invention can be used for emulsified flavors, an enteral hyperalimentation drug, a cosmetics milky lotion, etc. in preparation of an oil-in-water type emulsifying composition including emulsification foodstuffs, such as a cream kind, mayonnaise, a dressing, and pastes.

#### 0021

ExampleHereafter, although an example explains the operation aspect of this invention concretely, the technical range is not limited for this invention by these. 0022100 ml of 2-% of the weight solutions of example 1Thanh and Shibasaki, J.Agric.Food Chem., and beta-KONGU ricinine of the soybean obtained in accordance with the method of 24 and 1117 (1976) are adjusted to pH 7 and 70 \*\*, 0.01% per drained weight of papain (made by Sigma) was added, and the enzyme reaction was performed for 30 minutes. This 100 \*\* of reaction mixture is heated for 5 minutes, the enzyme was deactivated, the pH to 5 was adjusted, the centrifuged supernatant liquid was freeze-dried, and 200 mg of freeze-drying things were obtained. 13.3 ml of soybean oil was added to 40 ml of 0.5-% of the weight solutions (pH 5) of the freeze-drying thing, and the emulsified matter was prepared with the ultrasonic dispersion machine. This emulsified matter was centrifuged, it separated into the water layer and the emulsified layer, and emulsified layers were collected. The chloroform methanol (2:1) solvent washed this emulsified layer, it was air-dry, and 70 mg of solids (polypeptide mixture) were obtained. (3.5% of yield) 0023When the above-mentioned polypeptide mixture is analyzed by SDSelectrophoresis, the polypeptide of the molecular weights 5000 thru/or 6000 occupies 50% (fixed quantity by a densitometer). SDS - The band of these 5000 thru/or 6000 is transferred on a PVDF film from the gel which carried out electrophoresis, The place which investigated the N-terminal-amino-acid arrangement of these polypeptides by pro Dingxi KENSA, It was the polypeptide constituted from Asn-Phe-Leu-Ala-Gly-Ser-Gln-Asp-Asn-Val-Ile-Ser-Gln-Ile-Pro-Ser-Gln-Val-Gln-Glu-Leu-Ala-Phe- by both.

**0024**Turbidimetry estimated the emulsification power of the polypeptide mixture obtained in Example 2 and comparative example 1 Example 1 (J. refer to Agric.Food Chem. and the method of 26,716 (1978)). That is, 0.25 ml of soybean oil was added to 1 ml of solutions (pH 7) 0.05% of the weight, the emulsified matter was prepared with the ultrasonic dispersion machine, it diluted with the SDS solution 500 times 0.1%, and solution turbidity was measured (absorbance of 500 nm). As comparison, the same method as the above estimated the emulsification power of the pH 5

supernatant liquid dry matter (following comparative example 1) of the papain reaction mixture of Example 1.

0025Emulsification power (absorbance of 500 nm)

Polypeptide mixture (example 2) 0.35pH5 supernatant-liquid dry matter (comparative example 1) 0.18 polypeptide mixtures were high emulsification power. **0026**The polypeptide of 23 residue corresponding to the N-terminal-amino-acid arrangement of the polypeptide mixture obtained in example 3 Example 1 was compounded with the peptide synthesis machine. When the method (the same however, except having used 1 ml of solutions (pH 7) 0.02% of the weight) of Example 2 estimated emulsification power using this synthetic peptide, emulsification power (absorbance of 500 nm) was 0.75. Thus, 23 residue corresponding to the above-mentioned N-terminal-amino-acid arrangement was indispensable to high emulsification power.

## 0027

**Effect of the Invention**According to this invention, in the field of cosmetics, drugs, etc., the polypeptide, polypeptide mixture, and emulsifier which can be used when preparing an emulsion can be provided including foodstuffs.

#### 0028

Layout TableArray number: The length of 1 arrangement: Mold of 23 arrangement: Amino acid topology: Kind of straight-chain-shape arrangement: Peptide sequence Asn Phe Leu Ala Gly Ser Gln Asp Asn Val 1 5 10 Ile Ser Gln Ile Pro Ser Gln Val Gln Glu 11 15 20 Leu Ala Phe 21 23